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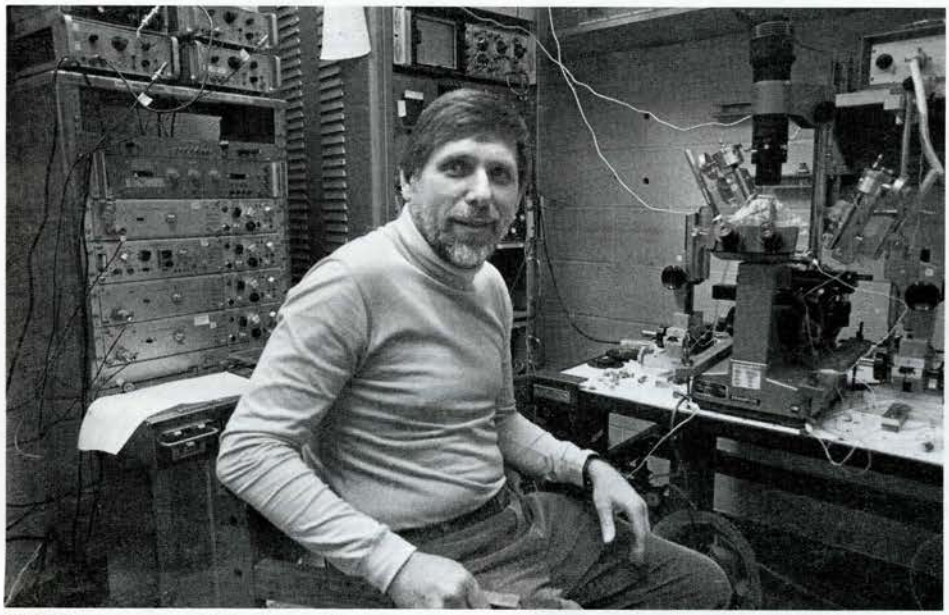
The cellular basis of life has been known for nearly 150 years, but much remains to be learned about how cells function. BUSM scientists study the workings of cell membranes in order to understand such disorders as Alzheimer's disease and cancer. See story on page 3.



Felix Strumwasser, Ph.D., is studying a model system as a means of understanding basic mechanisms of circadian oscillators. (Photo by Bradford F. Herzog)

BUSM researchers examine role of hormone abnormalities in hypertension

It is well established that abnormalities involving hormones can lead to hypertension.

An example is the steroid hormone aldosterone. Manufactured by the adrenal gland, aldosterone acts on the kidneys to promote salt retention.

Normally, once salt levels have reached some equilibrium, the gland stops making the hormone. But certain circumstances can keep the shut-down from occurring, said James C. Melby, M.D., a professor of medicine and pharmacology at BUSM and an internationally known endocrinologist.

"When the patient has an adrenal tumor or some other type of adrenal condition, the aldosterone can escape normal regulatory systems," explained Melby.

The ongoing flow of aldosterone that ensues means that the kidneys are constantly being stimulated to retain salt, and the ultimate result is hypertension. "It has been shown that aldosteronism is the cause of high blood pressure in between one-half and one percent of all hyper-

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Biological clocks may provide clues to manic depression, other ailments

The cycle of sleeping and waking, the regular rise and fall of body temperature, and the daily program of hormonal activity are all familiar examples of human circadian rhythms—patterns of physiological activity that repeat on a roughly 24-hour basis.

Scientists have long recognized the occurrence of these phenomena but only recently have biomedical researchers begun to explore the clinical relevance of these rhythms. For instance, a recent report shows that heart attacks occur three times more frequently at 9 a.m. than at 11 p.m. Similarly, a certain regimen of chemotherapy usually begun at 6 p.m. for patients with ovarian cancer is twice as likely to cause problems for the patient in terms of decreased tolerance and secondary infection as when the same treatment is begun 12 hours earlier.

In a normally functioning organism there are several physiological processes that oscillate in the circadian range. (An oscillator is a mechanism that causes any function to change its value regularly over time; the period of the oscillator is the length of time required for the process to complete itself and start again.)

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In humans, the body-temperature cycle is an example of a strong oscillator because it keeps close to a 25-hour cycle even if the individual is completely shut off from normal environmental time cues. Sleeping and waking, however, are governed by a weak oscillator because, after a few days or weeks of isolation, that cycle will break away to some extent from the body's temperature cycle.

This implies that if, for some reason, the internal body oscillators cannot stay in tune with each other, then they can produce irregular beats. According to Felix Strumwasser, Ph.D., a BUSM professor of physiology who is studying these rhythms on a cellular level, "There is a strong theoretical basis to believe that's what is happening in manic depressive cycles."

The drastic and devastating mood shifts of manic depression are rhythmic in individuals with the disease but the periods of cycles vary from person to person, from a few weeks to a year. Under a five-year grant from the National Institute of Mental Health, Strumwasser is using a model circadian system and lithium, the agent used most successfully to manage manic depressive symptoms, to get at the basic mechanisms involved in producing circadian oscillations.

"The real question is, how do nerve cells that work in units of milliseconds generate patterns of activity in an organism that last 24 hours and that are repeated every day," said Strumwasser.

To explore this question, Strumwasser and his former colleagues at California Institute of Technology several years ago discovered that a type of marine mollusk called a sea hare (*Aplysia californica*) has a 24-hour "clock" built into its eye that is completely independent of the external environment, although the timing of this clock can be entrained, or synchronized, by the environment.

The animal, which looks like a fist-sized snail without a shell, has eyes that produce optic nerve impulses at different rates at different times of day. Removed from the animal, the eye can be kept alive and active in a special medium for up to two weeks. Even if the isolated eye is kept in constant darkness, a record of its nerve activity over a two week period shows that the impulses continue to cycle normally, but slowly drift out of phase with the solar day.

"Since the eye can be studied apart from the influences of other circadian oscillators, we can look at physiological, pharmacological and biochemical markers for this particular oscillator to get insights on how it produces this regular rhythm," explained Strumwasser.

Strumwasser has found that lithium introduced to the culture medium can lengthen the duration of the impulse cycle from its normal 24 hours up to 33 hours, depending on the concentration used. Other agents, such as caffeine, lanthanum and manganese, have a similar effect. What all of these compounds have in common is the ability to interfere with calcium metabolism inside cells.

"The working hypothesis is that the circadian oscillator is sensitive to internal calcium changes and, therefore, one suspects that any disease that might influence calcium in cells is going to perturb (the rhythm) and possibly produce a set of symptoms that may not have anything to do at all with the primary symptoms a physician is dealing with," Strumwasser said. "The circadian oscillator itself does not have a special mechanism for stabilizing those agents inside a cell that can affect it, but rather depends on other body processes to regulate the agents."

Strumwasser is pursuing studies of how circadian oscillators are generated in nerve cells in terms of their molecular and biochemical mechanisms. He and former associate John C. Woolum, Ph.D., have found that if RNA metabolism is disrupted in cells either by x-rays or a drug such as actinomycin D, the rhythm of optic nerve impulses in the sea hare is abolished, although the eye remains otherwise healthy. "This was the first indication that circadian cycles in the eye appear to be dependent on a transcriptional event that occurs every day," said Strumwasser.

He postulates that there is a set of proteins that modulate a cell's membrane from the inside, across which nerve impulses are generated. Membrane modulation is dependent on new proteins being made and degraded every day. If no new protein is manufactured because of drugs or radiation, then they cannot continue to influence the function of the membrane and the rhythm can no longer be expressed.

The rhythm of nerve impulses in an isolated eye also can be sped up or slowed down depending on where in the cycle a perturbation is introduced. Cyclic nucleotides such as cyclic AMP (adenosine 3', 5'-monophosphate) and cyclic GMP (guanosine monophosphate) are agents that produce such phase shifts in the eye. "The evidence is fairly strong that cyclic AMP and GMP work through protein phosphorylation, a process by which the activity of a protein—whether in its role as a membrane channel or as an enzyme—can be turned on or off quickly. A pulse of cyclic AMP delivered to the eye produces a phase shift, which implies there is a change in the phosphorylation state of some key proteins to which the circadian oscillator is sensitive."

Strumwasser, senior research assistant Daniel P. Viele, graduate student Daniel Kohane and former colleague George Stone, Ph.D., are using radioactive methionine (a common amino acid unit of proteins) to try to pinpoint which newly synthesized proteins may be part of the circadian oscillator mechanism.

"We are looking at patterns of proteins being synthesized in the eye at different times of the circadian cycle, and noting especially if there are some proteins that are made at one time and not at another. If so, these may be important regulatory agents of the oscillator itself or of the membrane of the cell, or both."

(Other evidence has suggested that the circadian mechanism may be located in the neurosecretory cells of the eye, which make proteins to be exported to different areas of the brain.)

Much work, however, remains to be done at the basic molecular level to understand why lithium stabilizes manic depressive mood swings. Research on other biological preparations has demonstrated that lithium raises the levels of calcium inside different cells. Graduate student Mark Scanzillo is conducting experiments in Strumwasser's lab to stabilize internal calcium levels by using a calcium sequestering agent in order to determine if lithium will still be able to lengthen the circadian period of the eye.

Concluded Strumwasser, "Basically, the body has a 'slow program' of activity that impacts on probably every physiological function. Only now are we starting to look at the clinical relevance of the circadian programs and beginning to gain insight into the basic mechanisms involved."

—Caroline H. Lupfer

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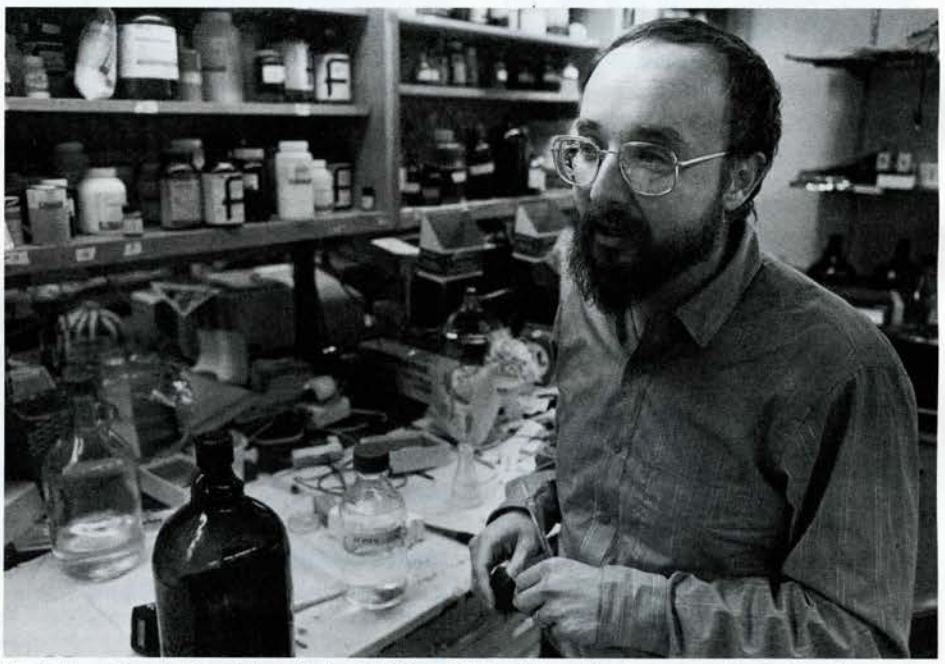
Cell membrane action may hold key to Alzheimer's and other disorders

Scientists have known for nearly 150 years that the cell is the basic unit of all life, but they still don't fully understand how it works. Researchers at Boston University School of Medicine are examining the intricate workings of cell membranes in an effort to better understand the normal aging process and such disorders as Alzheimer's disease and cancer.

Richard Fine, Ph.D., a cell biologist, is among the scientists at BUSM who are investigating the inner workings of normal cells, on the theory that these studies could offer insight into the functioning of abnormal cells. For a decade, Fine, a professor of biochemistry and an associate professor of physiology, has studied brain-, liver- and muscle-cells of laboratory animals to learn how normal cells work and how the cells are regulated.

"Once we know how a normal cell works, we will have an understanding that we can apply to cells that don't function properly," said Fine. Funded by the National Institutes of Health, Fine's research centers on a tiny cell membrane with a very regularly-shaped protein covering, the coated vesicle. Coated vesicles are derived from the plasma membrane, a lipid and protein envelope surrounding the cell, and appear to play a role in regulating the internalization of material into cells. Fine believes that the coated vesicle may be the transport vehicle that carries membrane proteins from place to place in cells.

A large part of his work is devoted to discovering the number of membrane transport steps in which the coated vesicle is involved. "Membrane proteins seem to be made primarily in one organelle (a specialized structure within a cell) and have to



In studies of coated vesicles, Richard E. Fine, Ph.D., is learning how normal cells function and where breakdowns may occur in such disorders as Alzheimer's disease and cancer. (Photo by Bradford F. Herzog)

be distributed to all the different organelles in the cell," he explained. "The coated vesicle selects the proteins to transport from one membrane to another and leaves behind proteins it does not want to transfer."

The process involves two major sorting decisions. From the millions of protein molecules in the membrane, the cell selects the correct molecules to move from one membrane to another. Then, after the molecules are removed from the membrane, the transport vesicle knows where to take those molecules within the cell.

Fine is most interested in the targeting problem: how the coated vesicle knows where to go in the cell. His lab has isolated coated vesicles in a highly purified form in brain-, liver- and muscle-cells. Although the coated vesicles from these three cell types all have the same type of coating, they carry different proteins. The protein determines the special function of each cell.

However, Fine explained, all three cell types appear to make the protein acetylcholinesterase, an important enzyme. The brain produces this enzyme to control a chemical neurotransmitter, acetylcholine, which is involved in the transmission of impulses between nerve cells. Muscle cells also produce this enzyme to prevent themselves from contracting too much. Scientists do not yet know why the liver manufactures the enzyme.

Acetylcholinesterase appears to hold a key to determining which of a cell's two major pathways are involved in the transport of proteins. One pathway leads from inside the cell to the plasma membrane; the other pathway leads from the plasma membrane to inside the cell. Fine said information about the pathways may contribute to an understanding of Alzheimer's disease, in which a particular pathway in the brain, the cholinergic system, appears to become disordered and fails to function.

Since aluminum and iron plaques have been found at the site of lesions in the brains of Alzheimer's patients, Fine is investigating the theory that such agents as toxic biochemicals, viruses and, perhaps, large protein molecules, can enter the brain and interfere with normal cell function. To discover the pathway these agents may take, he is studying in laboratory animals how iron, an essential molecule for all life, crosses the brain-blood barrier.

"Endothelial cells at the blood-brain barrier have receptors for transferrin, a protein in the blood that binds with iron," he theorized. "These receptors selectively take the transferrin with the iron into a vesicle. The vesicle then transports the iron-transferrin combination across the cell membrane, releasing it on the brain side of the cell membrane where it can be picked up by the brain cells that require it."

In experiments with rats, Fine has used radioactive transferrin to successfully demonstrate this selective receptor-mediated transport of the iron molecule from the blood into the brain.

To better understand how liver cells grow and are regulated, Fine is conducting studies with Nancy Bucher, M.D., a professor of pathology at BUSM and a leading expert in liver regeneration. Acetylcholinesterase and the coated vesicles in liver cells have played a significant role in their experiments. "Our ultimate goal," he said, "is to understand liver regeneration.

"If we can understand what makes the normal cells stop at a given state, I think we're going to learn a lot that would relate to our understanding of cancer," said Fine.

Bucher has shown that the quiescent liver of a normal rat, when stimulated to divide, becomes "incredibly active." Nonetheless, the liver stops regenerating when it reaches approximately normal size. Fine said, "We hope to isolate the proteins in the liver cell, put them into an artificial membrane, and study this process in order to understand how it is "turned on" and, very importantly, how it is "turned off."

Coated vesicles carry the newly synthesized membrane proteins, which are necessary to build new proteins during liver regeneration. Fine is investigating whether more coated vesicles are mobilized when more cells are produced.

In addition, Bucher has demonstrated that the hormone vasopressin is required for liver regeneration. When vasopressin is released in a normal liver, the liver cells recognize the hormone and respond to it, but the liver is not 'turned on' to grow. When two-thirds of the liver is removed, however, this metabolic hormone changes to a growth factor, said Fine.

Among those assisting with the liver research, Burton Dickey, M.D., an instructor in medicine, has succeeded in reconstituting and keeping active the vasopressin receptor so that it can be removed from its normal membranes and remain active. Colin Campbell, a graduate student, has had good results purifying phosphoinositide kinase, one of the key enzymes in liver regeneration. And Jordan Fishman, Ph.D., an assistant research professor of biochemistry, has discovered a protein in liver that appears to bind to two different hormone receptors and to prevent them from binding further hormones. "It seems to be an important regulator of this class of hormones," said Fine.

"We feel that we are making progress in our goals of characterizing this group of hormone receptors and regulators in metabolism and growth control," said Fine.

—Shirley B. Moskow

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Hormone abnormalities...
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tensives," said Melby.

Hypertension related to aldosteronism often can be cured by treating the adrenal problem that brought it on. There are cases of hypertension, however, where the symptoms mimic those of the aldosterone-linked condition but where there is no evidence of excessive amounts of the hormone.

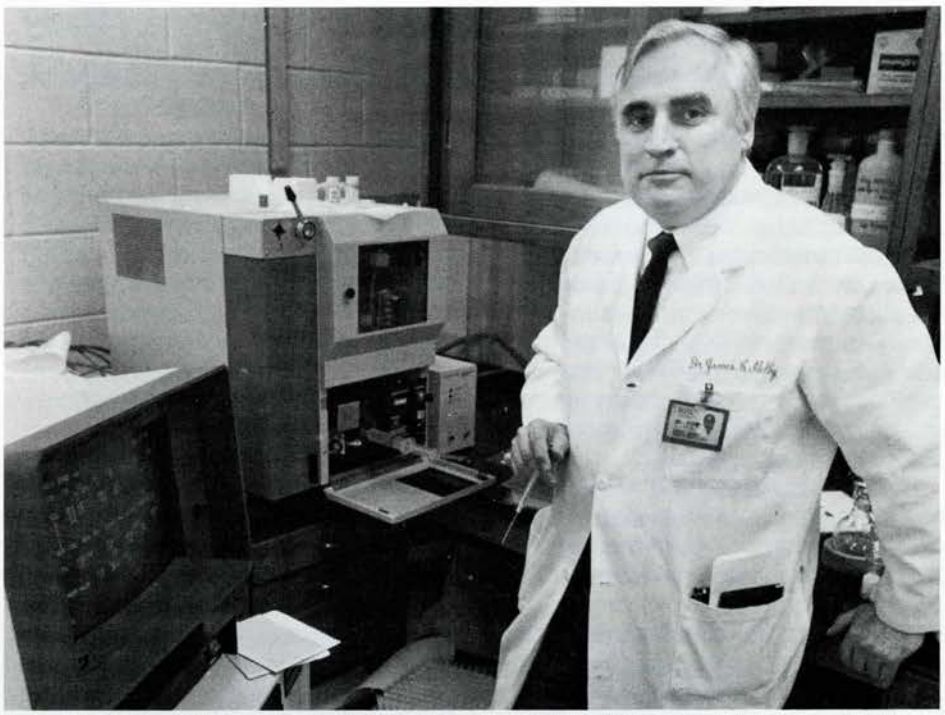
According to Melby, such cases suggest that hormones other than aldosterone play a role in hypertension. And after a quest spanning more than 15 years, he believes he has pinpointed one such hormone: 19-nor-deoxycorticosterone, or 19-nor-DOC. (The "19" refers to the position on a steroid hormone's carbon structure where a chemical compound called a methyl group can attach. The "nor" signifies, however, that this particular steroid hormone has no methyl group at that spot.)

In some ways, 19-nor-DOC is similar to aldosterone. It attaches to the same receptors in the kidneys, noted Melby, and presumably also boosts the tendency of those organs to retain salt.

But the production of the two hormones is not controlled in the same manner. In contrast to aldosterone, the levels of 19-nor-DOC do not rise and fall in response to increases and decreases in blood levels of the enzyme renin, which is manufactured in the kidneys. Instead, precursors of the hormone are made in the kidneys in response to signals from the pituitary gland, which is located in the brain.

Although the extent of 19-nor-DOC's role in hypertension is not yet known, the discovery that it does have a role has potentially vast implications.

An estimated 60 million Americans have blood pressure that is at or above the level considered worrisome: a systolic pressure of 140 and a diastolic of 90 (usually abbreviated to 140/90). Hypertension, moreover, has been identified by the Boston University-Framingham Heart Study as one of the three major risk factors for coronary disease, along with smoking and elevated blood levels of cholesterol. It also is implicated in an estimated 90 percent of strokes, and can cause heart failure, kidney failure and eye damage.



James C. Melby, M.D., is investigating how the abnormal production of a hormone may be the cause of hypertension in some individuals. (Photo by Bradford F. Herzog)

According to Melby, 19-nor-DOC was first identified in the 1940s. In the last 10 years, he suspected it might be a culprit in some cases of hypertension. But when he looked for it in blood samples drawn from rats that had been selectively bred to exhibit a specific type of hypertension—a form of the disease that is linked to the animals' salt intake—he couldn't find any evidence of the hormone.

Later, he discovered why not. Unlike aldosterone, he explained, 19-nor-DOC is not made in the adrenal gland. Instead, the gland produces precursors of the hormone. These then travel to various locations in the body, including the kidneys, and there are converted to 19-nor-DOC.

The fact that the hormone is made in the kidneys suggested that it should be possible to test the urine for evidence of elevated levels. Melby's group tried the technique in rats with salt-sensitive hypertension. As predicted, the animals had high levels of 19-nor-DOC.

"At that point, we started looking for 19-nor-DOC in urine samples from patients," he added, "and we found that it was significantly elevated in a large proportion of salt-sensitive hypertensives."

Since those early findings, Melby's group has discovered various ways in which excessive amounts of the hormone might be generated.

Crucial factors in the making of 19-nor-DOC are the pituitary gland and its well-known product, adrenal corticotropin hormone (ACTH). When the output of ACTH mounts—say, after an injury—it triggers an increase in the adrenal gland's production of the hormone cortisol. That hormone then

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travels to the injury site, helping to reduce the pain and to spur the arrival of infection-fighting white cells.

The group's research suggests that one cause of excessive levels of 19-nor-DOC is a breakdown in normal cortisol metabolism. That hormone is normally made through a series of steps that begins with cholesterol, and involves the creation of several intermediate substances before the appearance of cortisol itself. If something goes wrong with one of those steps, the end result may be the manufacture of other steroid hormones, including 19-nor-DOC.

In fact, Melby's group has pinpointed what appears to be just such a breakdown. It is related to a lack of 17*a*-hydroxylase, one of the enzymes involved in converting cholesterol to cortisol.

"If you have a 17*a* deficiency, you get a decrease in cortisol biosynthesis," he explained. "Since there's less cortisol to damp down the pituitary, you get this continuing flow of ACTH, and that stimulates production of other steroids, including the 19-nor-DOC precursors."

The 17*a* deficiency not only disrupts cortisol metabolism, but also inhibits the making of reproductive hormones. Thus, its symptoms include reproductive problems as well as hypertension. Men who lack the enzyme have poorly developed reproductive organs, said Melby, while women who are affected do not menstruate and cannot bear children.

The deficiency, which can be treated with substitute hormones, at best accounts for a miniscule proportion of hypertension cases. Melby estimates that no more than 30 Americans are affected by the condition. He also noted, however, that other things may go wrong with the cholesterol-cortisol pathway, leading to an excess of 19-nor-DOC.

To find out more about 19-nor-DOC's role in human hypertension, Melby and his associates are collaborating with investigators from Indiana and Louisiana on studies of large groups of hypertensives. At the same time, the BUSM investigators are looking for ways to treat or prevent hypertension related to 19-nor-DOC.

One drug that shows promise, said Melby, is tamoxifen, which is widely used to treat breast cancer. It appears to work by blocking production of the hormone. Another agent, developed in cooperation with investigators at the Massachusetts Institute of Technology, works by destroying one of the enzymes needed to create 19-nor-DOC.

In any case, Melby is confident that if further research shows that 19-nor-DOC plays a major role in hypertension, there will be no difficulty identifying agents to deal with the problem. He also believes that if the excessive output of 19-nor-DOC starts shortly after puberty, as he suspects, it may be possible to prevent the problem from causing hypertension.

"If we could screen adolescents for 19-nor-DOC," said Melby, "I believe we could interrupt this 19-pathway and prevent the individuals who are genetically prone to an excess of the hormone from ever being affected by it."

Other investigators involved in this research include research associate Sidney L. Dale, Ph.D., and George T. Griffing, M.D., an assistant professor of medicine.

—Richard P. Anthony

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